THE AMMONIACAL FERMENTATION OF URINE.

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The fact is well known that urine undergoes putrefactive changes when it is allowed to stand for a considerable length of time at the ordinary temperature. In following these in the order of their occurrence we find that the clear urine first becomes faintly, then heavily clouded, and finally, after several weeks standing, it clears with the formation of a considerable quantity of a grayish, more or less granular, sediment. The color of the cleared urine is always several shades darker than that of the fresh liquid. A further examination shows that its acid reaction is converted into an alkaline one simultaneously with the occurrence of the clouded appearance, and with this change in its chemical reaction the odor becomes penetrating and ammoniacal in character. The source of the ammonia was explained by the discovery of urea, and later investigations made by Prout showed that the urea was converted into carbonate of ammonia by a process of hydration. By "ammoniacal fermentation of urine," therefore, is meant that change in urine by which its urea is transformed into carbonate of ammonia.

The careful chemical investigations that have been made during the past two centuries for the purpose of determining the cause of this transformation of urea into carbonate of ammonia have been negative in their results. Van Helmont, in the seventeenth century (1682), believed the cause to be the action of some putrefactive ferment, the exact nature of which he did not know. He was supported in this theory by other investigators following him; but a satisfactory explanation of this phenomena was not made until the time of Pasteur, who showed that the "ammoniacal fermentation" was due to the presence of an organized ferment (micro-organisms), and thus he transferred the line of investigation of this subject from a purely chemical to a biological basis.

The bacteriological investigations of this subject by Pasteur, Van Tieghem, Miquel, Leube, and others during the last thirty years have been so confirmatory that no further evidence is necessary to establish the theory of an organized ferment advanced by Pasteur. I have repeatedly preserved urine for several months in apparently a perfectly fresh condition by first sterilizing it by discontinuous heating for several days at a temperature of 65° C., after which it was continuously exposed to a temperature most favorable for the development of ammoniacal fermentation; also by placing it unsterilized in a temperature below that necessary for the development of bacteria. The results of many carefully conducted experiments warrant the statement that without the development of certain bacteria urine will remain unchanged although subjected to all the other influences necessary for this decomposition. As experiments have shown that the urine within the healthy bladder of people not suffering from bacterial disease is germ free, it is evident that the organisms producing this decomposition are to be sought for among the external surroundings or in the urethral canal, where they have entered either by accident or where, when once introduced, they remain in accordance with some as yet unknown law of localization. Their presence in this canal is shown from the fact that urine passed directly into sterile flasks without external contamination will undergo decomposition; but if the urethral canal is previously disinfected with some antiseptic solution it will remain unchanged for an indefinite time.

Biological History.

In 1860 Pasteur (1) showed that in decomposing urine the transformation of the urea into carbonate of ammonia was due to a micrococcus which he found and designated as *Torula ammoniacale*. A few years later Van Tieghem (2), in his inaugural thesis, presented to the "Faculty of Sciences of Paris," considered the cause of ammoniacal fomentation to be a micrococcus (spherical globules), which arranged themselves in long chains (streptococci).

Twelve years later (1876) Pasteur (3) recognized streptococci in decomposed urine, but says: "They must not be confounded with the ferment of urine, which they resemble in the diameter of their grains. The ferment of urine is formed of *couples de grains*" (diplococci). Whether the streptococcus of Van Tieghem and the diplococcus of Pasteur are identical or not we will not attempt to decide. The crude condition of bacteriology at that time, in

the presence of subsequently developed facts, will admit of their being considered identical species, notwithstanding the discrepancies in the original descriptions. Professor Leube and P. Miquel have considered them as belonging to the same species.

In 1879 P. Miquel (4) isolated a rod-shaped germ from decomposed urine which, when introduced into sterile urine, would cause it to undergo ammoniacal fermentation. He gave it the name *Bacillus urea.*

Six years later (1885) Professor W. Leube (5) published an article upon "The Ammoniacal Fermentation of Urine," in which he describes three rod-shaped germs and one micrococcus isolated from urine and a sarcina from the saliva that would transform urea into carbonate of ammonia. The rod-shaped germ first described he called *Bacterium urea.* The other two are not specifically named other than by number. The micrococcus he considers to be the same as the one studied and described by Pasteur and Van Tieghem.

Flügge (6) describes a micrococcus that was isolated in his laboratory and which produced the same effect on urine as the micrococcus described by Pasteur. On account of its power to liquefy gelatine it was called *Micrococcus urea liquefacinas.*

In 1889 P. Miquel (7), in an article on "Ferments of Urine," describes six bacilli (Bacillus urea φ, B. u. β, B. u. γ, B. ε, B. δ, and B. ρ), five micrococci (Micrococcus urea φ, M. u. β, M. u. γ, M. u. δ, and M. u. ε), and one sarcina (Sarcina φ), which would transform urea into carbonate of ammonia. The *Bacillus urea* β is the same as that described by him in 1879, and he considers it the same as the *Bacterium urea* of Leube. He quotes from several investigators who have conceded to him the priority in the discovery of this bacillus The micrococcus urea φ is the same as the micrococcus urea of Leube, and supposed to be the same as the micrococcus studied by Pasteur and Van Tiegheur. The sarcina is the same as that described by Leube. To summarize, we have as organized ferments of urine already described six micrococci (Pasteur's, Flügge's, and Miquel's), seven bacilli (eight if Miquel's *Bacillus urea* β and Leube's *Bacterium urea* are not indentical), and one sarcina that are more or less active in the transformation of fresh into ammoniacal urine.

In considering the source of the organisms, we find that Pasteur, Van Tieghem, and Leube isolated their germs from decomposed urine. Flügge does not give the material from which he obtained his mic. urea liquefacians. Miquel found the germs he described

in the atmosphere, soil, and water. Pasteur also found his *Torula ammoniacale* among the germs isolated from the atmosphere.

In view of these facts, I have confined my investigations thus far principally to those forms of bacteria that are to be found within the healthy urethral canal. The object of such a limit was to determine, if possible, whether or not there are certain bacteria that are constantly present in this canal which act as exciting agents in the transformation of fresh into ammoniacal urine. If this question can be definitely settled it may be effectual in explaining the causation of certain cases of cystitis which are rapidly developed after the introduction of bougies and catheters. Dr. Ricard (8) finds that if these instruments are made aseptic that their introduction is harmless, providing the urethral canal is not infected; but if the urethral canal is already infected, it must previously be washed with a saturated solution of boric acid in order to avoid a subsequent more or less acute cystitis. It seems reasonable to suppose that if germs are found constantly within the healthy urethra that are active in prodncing ammoniacal urine that this canal as well as the instruments should always be thoroughly disinfected before any operation is attempted.

Methods Employed in Collecting and Examining Urine.

The methods employed both in collecting the fresh urine and in its subsequent examination are of much importance and worthy of careful consideration. The following precautions were rigorously adhered to in collecting the fresh liquid: The glands and external urinary meatus were carefully disinfected with either a 5 per cent. solution of carbolic acid or a 1–1000 solution of corrosive sublimate. The urine was passed directly into a sterilized Erlenmeyer flask, the mouth of which was previously flamed and the cotton-wool stopper replaced immediately after urination. Care was also taken to avoid dust and currents of air. A few cubic centimeters were immediately removed from the flask by means of a sterile pipette for a preliminary chemical examination. Only normal acid urine was retained for the bacteriological examination.

For the isolation of the germs the well-known Koch gelatine-plate method was employed. Esmarch rolls were at first tried, but on account of the presence of liquefying germs they were abandoned. In several cases a plate culture was made from the fresh urine by adding from ¼ to 1 c. c. of it to a tube of liquid gelatine by means

of a sterilized pipette, and after thoroughly mixing, pouring the gelatine upon sterile glass plates. These were kept at a temperature of 65 to 75° F. These plates developed from one to four colonies, occasionally more, often none at all, showing how comparatively few bacteria there are in freshly voided urine. The flasks were allowed to stand at the ordinary temperature (65° to 75° F.) for from 24 to 48 hours, when the urine became clouded and either neutral or alkaline in reaction. A microscopical examination at this time showed a greater or less number of bacteria present. Plate cultures in gelatine were then made. One small platinum wire loop (holding from $\frac{1}{60}$ to $\frac{1}{50}$ c. c.) of the fermenting urine was added to a tube containing about 10 c. c. of liquid gelatine. After thoroughly mixing, from 2 to 4 loops of the first gelatine were added to a second tube of gelatine. The gelatine was poured on sterile plates. (These were carefully protected from external contamination by means of flamed bell jars. Sterile "double-dishes" were also used.) They were kept at a temperature varying from 65° to 75° F. The number of loops taken for the first tube of gelatine depended upon the number of germs seen in the microscopical examination. When the germs were exceedingly numerous the first dilution was made in a tube containing about 7 c. c. of sterile distilled water. The first plate usually developed innumerable colonies after 24 to 48 hours. The second plate as a rule developed from 50 to 300 colonies. As soon as they were sufficiently developed they were examined both *macroscopically* and *microscopically* and a subculture made in sterilized urine and gelatine from a colony of each apparently different form. The plate cultures were repeated after the urine had reached its maximum degree of alkalinity, and again after it had been standing for from 2 to 3 weeks. In a few cases parallel plates in agar were made, but no germ was found to develop in this medium that did not grow on the gelatine. The subcultures were carefully examined, and all those in which the urine culture became alkaline were retained for more careful study. By this process I have isolated from urine about 12 forms of bacteria, mostly micrococci, of which five, on account of their property of producing ammoniacal fermentation in urine, will receive a somewhat detailed description. Of the remaining forms, one was a strepticoccus which was present in every specimen of urine examined, two were bacilli, and the remainder were micrococci. The bacilli were very rarely found.

Culture Media Employed in Testing the Reaction of These Germs.

For a preliminary examination of these germs the ordinary culture media were employed, but in order to test their power to transform urea into carbonate of ammonia, media containing that substance were necessarily resorted to. Of those sterilized normal urine seemed to be the most practical, as well as to afford a sufficient test for the property of the germs in question. Other media, such as acid-beef-infusion-peptone and gelatine plus urea, were also employed. Of the ordinary media, nutritive gelatine was the most important from a differential standpoint.

(1) *Sterile urine:* This was prepared by distributing fresh acid urine in sterile culture tubes, about 10 c. c. in each tube. As the urea is broken up by a high temperature the tubes were placed in a large water-bath and heat to a temperature of 65° C. for two hours each day for four consecutive days. Their sterility was tested by allowing them to stand for several days in an incubator at a temperature of 36° C. The urine remained in every instance clear and acid in reaction.

(2) *Acid-bouillon plus urea:* This was prepared by macerating 100 grams of finely chopped beef (freed from fat) in 200 c. c. of distilled water. After standing for from 18 to 24 hours in a cool place it was strained through a coarse linen, and to the simple beef-infusion ½ per cent. sodium chloride and 1 per cent. peptone were added. It was then boiled for thirty minutes, cooled, filtered, and ½ per cent. urea added. The liquid was then distributed in sterile culture tubes and sterilized by heating at 65° C. for three hours each day for four consecutive days. It remained clear and acid after standing for several days at a temperature of 36° C.

(3) *Acid gelatine plus urea:* This is prepared precisely in the same manner as the acid bouillon, with the addition of 10 per cent. gelatine, which is added before the beef-infusion is boiled. The urea is added to the liquid gelatine after it is filtered. It is preserved in sterile cotton-plugged test tubes and sterilized by the same method as the bouillon, after which it remained clear and acid in reaction.

Description of New Germs.

In the light that we already have concerning this class of organisms and in the present unsettled condition of bacteriological classification, it seems more desirable to omit the assigning of specific names to the micrococci about to be described, but simply differ-

entiate them with reference to their morphological characters and their growth in certain culture media. They will, therefore, be designated by the first letters of the alphabet, printed in small capitals, as *micrococcus* A, mic. B, etc. In comparing the morphological and cultural characters of these germs with the description of the micrococci described by Pasteur, Flügge, and Miquel I have failed to find sufficient resemblances to establish the identity of any of these micrococci with those heretofore described. Their effect upon urea in nutrient solutions is, however, practically the same.

Micrococcus A.—A micrococcus varying in diameter from 0.6 μ to 1.2 μ; average, 0.9 μ. When grown in bouillon the cocci appear most usually in small clumps and in the form of diplococci. There seems to be a marked disposition in the cocci to be closely united in pairs. In acid bouillon they are frequently observed united in short chains.

Agar (surface growth) at 36° C.—After 24 hours round, convex, grayish colonies appear with a glistening surface and sharply defined border. When they are separated by a distance of one-half inch or more they are about 1 millimeter in diameter. After growing for several days the colonies become opaque, whitish, but otherwise unchanged in appearance. The colonies are quite brittle. The growth in the condensation water is quite vigorous, giving it a clouded appearance.

Gelatine (needle culture).—In nutrient gelatine the growth is quite vigorous at 70° F. Along the needle track, after 24 hours, a grayish, opaque, somewhat granular line appears, which consists of crowded colonies. At the surface the growth is more vigorous, forming an elevated glistening band around the needle puncture. This may be due to the greater number of germs collected there. On the second or third day the gelatine begins to liquefy along the entire length of the needle track. At first the liquefaction is generally more rapid on the surface, giving a cone-like appearance to the liquid portion, the base being at the surface (Pl. 1, Fig. 1). Very often the liquefaction is in the form of a cylinder. The entire mass of gelatine is liquefied in from 8 to 20 days. In a higher temperature (75° to 80° F.) the growth is more rapid. The liquid gelatine holds in suspension fine granular masses of germs $\frac{1}{10}$ to $\frac{1}{4}$ millimeter in diameter. A considerable quantity of a grayish sediment collects in the bottom of the tube; also a fine granular pellicle forms over the surface. Both the pellicle and sediment are very viscid.

The liquefied gelatine will not set, showing that it has become peptonized.

In alkaline bouillon the growth is very feeble, the liquid becoming barely clouded, even after several days standing. In acid bouillon the growth is at first also feeble, but on the second day it is very much increased, the liquid is quite clouded, and a granular pellicle forms over the surface. The acid reaction is not appreciably changed. In sterile milk at 36° C. this germ coagulates the casein after about two weeks standing. It grows very vigorously. The reaction remains slightly acid. A slight sour-milk odor is emitted.

Sterile urine at 36° C. This germ multiplies very rapidly in this liquid. After 18 hours it becomes quite clouded with the formation of a grayish sediment. Its acid reaction is changed to a feebly alkaline one, which becomes very strong after 36 to 48 hours. The odor is penetrating, resembling that emitted by ammonia water. In acid bouillon plus urea at 36° C. this germ grows very rapidly. After 24 hours the liquid is very clouded, with considerable sediment formed in the bottom of the tube. The reaction is changed to a strong alkaline one. A slight ammoniacal odor is emitted. In the acid gelatine plus urea the growth is more vigorous and the liquefaction more rapid than in the ordinary gelatine. With the liquefaction its reaction is changed to a strong alkaline one. The liquid gelatine becomes perfectly cleared, with a somewhat viscid sediment.

This germ is easily stained with the aniline dyes. It does not take the Gram stain.

Micrococcus B.—A micrococcus varying from 1.0 μ to 1.8 μ in diameter; average, 1.4 μ. It occurs single, in pairs, and small clumps. It is occasionally seen in short chains, especially when grown on agar or gelatine.

Agar (surface growth) at 36° C.—The growth of this germ on agar resembles in its general appearance that of the micrococcus A. It differs from that germ in two respects: (1) The growth on the surface is very viscid. (2) The condensation water remains clear, with a grayish, somewhat viscid growth at the bottom.

Gelatine (needle culture) at 75° F.—After 24 hours a grayish granular line appears along the needle track. At the surface the growth is more vigorous, spreading and liquefying the gelatine in a thin layer over the entire surface. The growth along the needle track does not increase, but the gelatine gradually liquefies from the

surface downward until from one-half to two-thirds of the gelatine has become liquid. The liquefaction does not extend beyond that point (Pl. I, Fig. 2). A more or less thick, whitish membrane forms over the central portion of the surface, rarely forming a complete pellicle. A layer of grayish, viscid growth forms over the surface of the non-liquefied gelatine. The liquid portion holds in suspension grayish granules, which are occasionally so fine as to give a uniform clouded appearance to the liquid gelatine. The liquefaction is completed in from one to two weeks.

In bouillon at 36° C. the growth is very feeble. After 24 hours the liquid is very faintly clouded. In from two to four days a somewhat stringy growth appears in the upper part of the liquid; also granular masses, varying from 1 to 2 millimeters in diameter. These are quite viscid. In acid bouillon, after several days a few granular masses are formed on the surface; also a whitish deposit on the sides of the tube at the surface of the liquid.

In sterile milk this germ, after about two weeks, coagulates the casein, leaving a clear supernatant liquid.

Sterile urine at 36° C.—This germ grows rather feebly in urine. After 24 hours the liquid is very faintly clouded and neutral in reaction. After 48 hours it is strongly alkaline and clouded with the formation of a small quantity of sediment. In acid bouillon plus urea the growth is not vigorous. After 24 hours the reaction is feebly alkaline. At the end of two to three days the reaction is strongly alkaline and the liquid quite cloudy—a slight penetrating odor. In acid gelatine containing urea the growth is more vigorous than in the ordinary gelatine. The liquefied gelatine is strongly alkaline in reaction.

This germ is easily stained with the aniline dyes. It takes the Gram stain.

Micrococcus C.—A micrococcus varying in diameter from 0.8 μ to 1.5 μ; average, 1.2 μ. It occurs single, in short chains, and small clumps. There are also diplococci forms.

Agar (surface growth) at 36° C.—On this medium the growth does not differ appreciably from that of micrococcus A.

Gelatine.—This germ liquefies gelatine very slightly, so that the growth, which at first is upon the surface, after several days becomes slightly depressed, never more than one to two millimeters. The growth along the needle puncture is whitish, opaque, viscid, and quite thick. The colonies along the needle track are very small. They do not liquefy the gelatine. (Pl. I, Fig. 3.)

In bouillon the growth is very feeble; after 24 hours the liquid becomes faintly and uniformly clouded. There is no further development. Sterile milk inoculated with this germ and kept at a temperature of 36° C. becomes thickened in the lower half of the tube after about six days. There is no separation of the casein from the aqueous portion; no odor; slight acid reaction.

Sterile urine.—In this liquid the growth is feeble. On the day following its inoculation the reaction is faintly alkaline, the liquid barely clouded. On the third day there is a thin granular deposit on the sides of the tube, and the reaction is strongly alkaline, ammoniacal odor. In acid bouillon plus urea at 36° C. the liquid becomes clouded on the day following the inoculation, with the formation of a considerable quantity of sediment; strong alkaline reaction, penetrating odor. After ten days standing it becomes clear, with a sediment. It remains strongly alkaline. In acid gelatine plus urea the growth is very feeble; no liquefaction.

It is easily stained by the ordinary methods. When stained according to Gram a great majority of the cocci take the brown stain perfectly. A few (about one in fifteen) retain the blue stain. These are of the maximum size (1.5 μ).

Micrococcus D.—A micrococcus varying in diameter from 0.7 μ to 1.2 μ; average, 0.9 μ. It grows most usually in small clumps and in pairs. It is very rarely seen single.

Agar (surface growth) at 36° C.—When not crowded the isolated colonies, after 24 hours, are from 1 to 1½ millimeters in diameter, round, flat, with a smooth border and sharply defined margin; A considerable growth in the condensation water. The colonies at first are grayish white, glistening, but after several days they become a light cadmium yellow. The size is very slightly increased. When grown on agar containing 5 per cent. glycerine the growth is more feeble, but the color more intense.

Gelatine (needle cultures).—Along the needle track the colonies develop after 24 hours as minute grayish points. After several days they attain to a diameter of ⅛ to ¼ millimeter, spherical, granular, yellowish. On the surface the growth is spreading, glistening, of a deep cadmium yellow. The growth about the needle puncture appears in more or less concentric bands. The outer bands also have a radiating appearance, produced by alternate lighter and darker lines extending outward from the more central portion. After about two weeks the growth becomes slightly depressed below the surface (Pl. I, Fig. 4).

In bouillon the growth is feeble. After 48 hours, at 36° C. the liquid becomes clouded in the upper portion of the tube, with a small quantity of a grayish deposit formed on the side of the tube. It produces no change in the appearance of milk.

Sterile urine.—In this liquid the growth is abundant; the liquid is heavily clouded after 24 hours; the reactions strongly alkaline. After from two to three days a considerable quantity of sediment is formed in the bottom of the tube. This germ is the most rapid in its transformation of acid into alkaline urine of any of the germs here described. Under its influence acid urine will become decidedly alkaline in eighteen hours. In acid bouillon containing urea the growth is vigorous. In 24 hours the liquid is very cloudy and strongly alkaline in reaction. In acid gelatine plus urea the growth is more feeble than on the ordinary gelatine. It takes the Gram stain.

Micrococcus E.—A micrococcus varying from 0.9 μ to 1.5 μ in diameter; average, 1.2 μ. It occurs most usually in small clumps and in pairs. It is frequently found single, often in short chains.

Agar (surface growth) at 36° C.—After 25 hours quite fleshy, round, convex colonies appear, 1 millimeter in diameter when not crowded. They are of a grayish yellow color, apaque. After several days the growth becomes a much deeper yellow. The condensation water is clear, with a viscid, flaky growth at the bottom. The surface growth is not viscid.

Gelatine.—The growth in this medium resembles that of the micrococcus B. It differs from it in the color of the membrane and sediment, which are cadmium yellow. (Pl. 1, Fig. 5.)

In bouillon the growth is feeble. It does not produce any apparent change in the appearance of milk.

Sterile urine.—The growth in this liquid is very slow. On the second day after inoculation it becomes feebly alkaline, with the formation of a granular deposit on the sides of the tube. In acid bouillon containing urea the growth is more vigorous. After one day, at 36° C. the reaction becomes strongly alkaline, emitting a penetrating odor. It liquefies acid gelatine containing urea very slowly. The liquefaction does not extend more than three to five millimeters below the surface. The liquid gelatine is strongly alkaline in reaction. This germ is readily stained by the ordinary methods. It takes the Gram stain.

The characteristic properties of these micrococci, especially their

reaction on nutrient gelatine, are more or less modified by cultivation. Their power to break up urea, however, does not seem to be diminished.

I have examined, after the methods previously described, twelve specimens of urine. Of these seven contained only the micrococcus A, two the micrococcus C, one the miccrococci A and B, one the micrococci B and D, and one the micrococci A, C, and E. Eight of the specimens were taken from the same individual, six of which contained (of the germs described) the micrococcus A only, and two the micrococcus C. The other four specimens were obtained from four other individuals, one of which contained only the micrococcus A.

In addition to the above, one specimen of urine from a person suffering with cystitis was examined. The urine was clouded, strongly alkaline in reaction, and contained a considerable quantity of crystals, principally triple phosphates, pus cells, and amorphous salts. A large number of micrococci were seen in a cover-glass preparation. The bacteriological examination showed that a large number of these were the micrococcus A. Thus far this is the only specimen of urine from an authentic case of cystitis that I have been able to obtain in a condition suitable for a bacteriological examination.

I have also isolated several germs from external sources that are equally as active in the production of ammoniacal fermentation as those already described. Two of these, a bacillus and micrococcus, were obtained from decomposed urine that was previously sterilized and then exposed to the atmosphere of the laboratory by removing the cotton-wool stoppers from the tubes containing it. The sterile, acid urine became clouded and alkaline in from three to five days. A second bacillus was obtained by inoculating a tube of sterile urine with several drops (about 1 c. c.) of Potomac water and allowing it to stand in an incubator, where it became clouded and alkaline in twenty-four hours. The bacillus was isolated from the other germs by means of plate cultures. A micrococcus was also found in saliva which is arranged in fours (tetracoccus), that is very active in transforming urea into carbonate of ammonia. These germs have, as yet, not been carefully studied, and their isolation is mentioned here only to illustrate the general distribution of bacteria possessing this property as well as to confirm similar statements made by other workers in this line.

Soluble Ferments.

In the last decade Musculus (9) announced the discovery of a soluble ferment which he obtained from ammoniacal urine that would, when introduced into fresh urine, transform its urea into carbonate of ammonia. The name *urase* was proposed for this substance, as it was supposed to belong to the same class of ferments as ptyaline diastase, etc. The method of isolating this ferment was reported by Musculus to be one of simple precipitation. The results, however, that have been reported by others who have attempted to isolate this substance have been, so far as I can learn, negative in character. P. Miquel, after a long series of experiments, in many of which the method of Musculus was carefully carried out, concludes with Prof. Leube, that the existence of a soluble ferment in urine is not sufficiently proven to be accepted without further demonstration. Thus far I have made no experiments for the purpose of isolating a soluble ferment. Whether there is such a ferment or not will, together with many other interesting questions that these preliminary investigations have suggested, have to remain unanswered until other and more extended investigations shall be made.

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PLATE I.

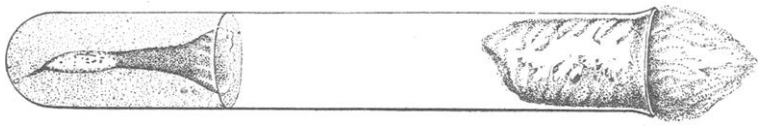

FIG. 1.

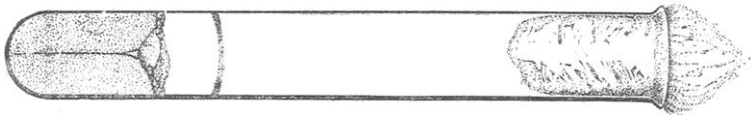

FIG. 2.

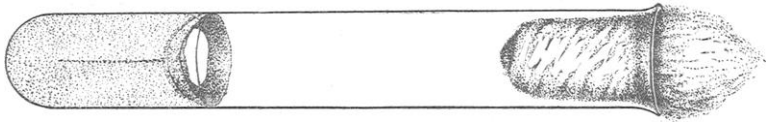

FIG. 3.

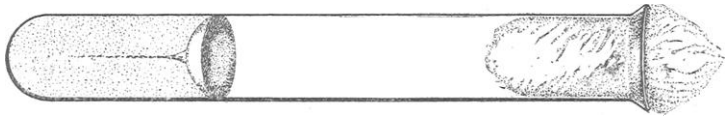

FIG. 4.

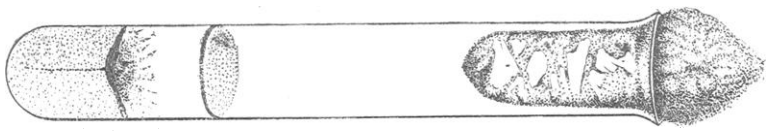

FIG. 5.

Description of Plates.

Plate I.

Gelatine needle cultures of the micrococci described.

Fig. 1. Micrococcus A—Culture seven days old.
" 2. " B " " "
" 3. " C " six weeks old.
" 4. " D " " "
" 5. " E " " "

Plate II.

Drawings made from cover-glass preparations of urine cultures, one day old, of the micrococci described. The preparations were dried, passed three times through a flame, and stained for a short time with alkaline-methylene-blue. The position of the germs in the field was determined by means of Abbé's camera lucida, Zeiss 2 mm. apochromatic lens, No. 4 eye-piece.

Fig. 1. Micrococcus A.
" 2. " B.
" 3. " C.
" 4. " D.
" 5. " E.

PLATE II.

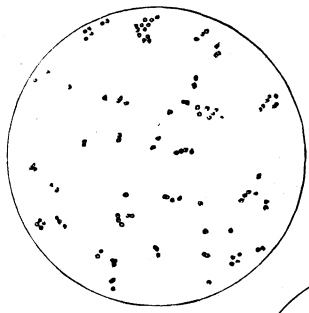

Fig. 1

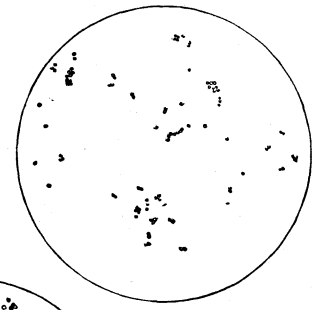

Fig. 2

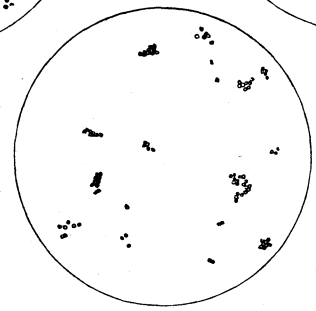

Fig. 3.

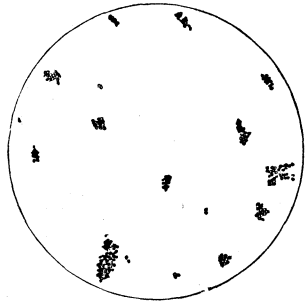

Fig. 4

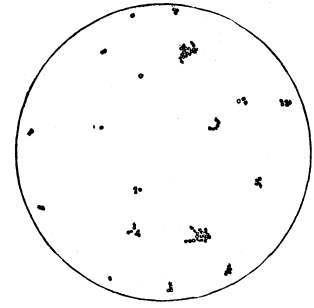

Fig. 5